Claim 20 (New Claim): The compound of claim 4, wherein Z^1 is N, Z is CH, and p = q = 1.

Claim 21 (New Claim): The compound of claim 20, wherein p = 1 and q = 0.

REMARKS

Claims 1-18 are under prosecution. In the present Office Action, Group I of claims 1-18 (drawn to compounds of Formula I wherein one of X and Y is N, the other is CH, M is a moiety having the structure shown in Formula II, the compositions and methods of use) were examined. In a telephone conversation with the Examiner on February 14, 2003, the undersigned Attorney-of-Record provisionally elected, with traverse, the claims of Group I for examination purposes. This Response affirms that election with traverse. Applicants, however, continue to believe that all claims 1-18, as filed, form part of one and the same invention. Applicants believe that when there is a linking claim encompassing the scope of all the processes, uses, kit and compounds, it is inappropriate to restrict the invention into these various inventions. Applicants also believe that due to such commonality a complete examination of claims 1-18 would not cause undue burden. Applicants further believe that the same art search will most probably apply to the alleged separate inventions, and respectfully submit that the restriction is improper.

Under the statute "two or more independent and distinct inventions.... in one application may.... be restricted to one of the inventions." Inventions are "independent" if "there is no disclosed relationship between two or more subjects disclosed" (MPEP 802.01). The term "distinct" means that "two or more subjects as disclosed are related.... but are capable of separate manufacture, use or sale as claimed, and are patentable over each other" (MPEP 802.01). However, even when patentably distinct inventions, restriction is not required unless one of the following reasons appear (MPEP 808.02):

1. Separate classification
2. Separate status in the art; or
3. Different field of search.

In the present application, Applicants believe that the Examiner has not established a clear reason to establish the existence of any of the above 1-3 situations. Reconsideration and withdrawal of the restriction requirement are, therefore, respectfully requested.

Claims 1-4, 6, 7, and 10-18 were objected to as being an improper Markush grouping. Applicant believes that the present amendment has addressed that concern of the Examiner. Withdrawal of the objection is, therefore, respectfully requested.

Claims 1-4, 6, 7, 10-15 and 18 were rejected U.S.C. §112, first paragraph, "because the specification, while being enabling for preparation of compounds wherein M is piperidine or piperazine, does not reasonably provide enablement for preparation of compounds wherein M is other than the above specified functional groups". Page 4 of the Office Action. In this amendment, Applicant has defined M to be Formula II, however, amending, at the same time, said Formula II to include piperazine, piperidine and pyrrolidine moieties with the point of attachment being top or bottom of said piperidine and pyrrolidine. See amended claim 5, and the new claims 19-21. This is being done to make sure that the pyrrolidine compounds (in the Examples) are also covered in the scope of the elected claims. Applicant believes that this satisfies the Examiner's concerns without straying away from the election. Withdrawal of the §112, first paragraph rejection is, therefore, respectfully requested.

Claims 13-15 and 18 were objected to for the listing of various diseases associated with the H_3 receptor. The objection is reasoned this way: "However, in a review article about histamine-H3 receptor, provided by applicants, Leurs and Timmerman stated the H3-receptor agents might be useful for *only* airway and gastrointestinal disorders (p 162, Drug Res. 1992)" Page 5 of the Office Action, (*italics* added by Applicant). Applicant would like to respectively point out that the word "only" does not occur in that cited passage. The correct quote on that page 162 reads: "It is already apparent that H_3-receptor agents might provide new therapeutics for airway and gastrointestinal disorders. Moreover, several findings also indicate a possible application in the CNS. However, at this time these

suggestions are still preliminary and will have to be validated by extensive research ...". Furthermore, H. Stark *et al*, *Pharmazie*, 52 (1997) 495-500, page 495 state: "Several therapeutic indications for H_3-receptor antagonists have been proposed for various diseases or conditions of the CNS..., e.g., memory and learning defects..., cognitive and sleep disorders..., and epilepsy." Applicant has enclosed a copy of the Stark article as Exhibit 1. Thus, the art recognizes the utility of H_3-receptor antagonists for the treatment of various diseases and not just for treating airway and gastrointestinal disorders. Withdrawal of the objection is, therefore, respectfully requested.

Claims 1,11, 12, 14, 16 and 17 were rejected under 35 U.S.C. §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Page 6 of the Office Action. The Examiner objected to usage of terms such as "including", and "substituted or unsubstituted" in the claims, certain duplicate claims, and claim 14. This amendment cancels or amends those terms/claims. The amendment for "substituted" has support in the specification on page 10, lines 7-9. Withdrawal of the §112, second paragraph rejection is, therefore, respectfully requested.

An unintended typographical error in the serial number of the priority application on page 1 of the specification stands corrected.

There being no other rejection pending, Applicant believes that the claims, as amended, are patentable over the art. Such an action is earnestly requested. If the Examiner has questions, the Examiner is invited to contact the undersigned.

June 26, 2003

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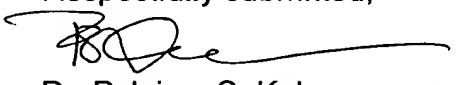
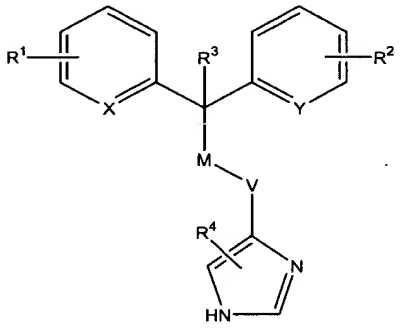
Respectfully submitted,



Dr. Palaiyur S. Kalyanaraman
Attorney for Applicants
Reg. No. 34,634

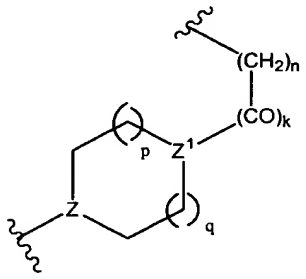
Marked Up Version of Amended Claims
(Added terms are underlined and deleted terms are in brackets)

Claim 1 (amended): A compound, or [including] enantiomers, stereoisomers and tautomers thereof, or pharmaceutically acceptable salts or solvates of said compound, with said compound having the general structure shown in Formula I:

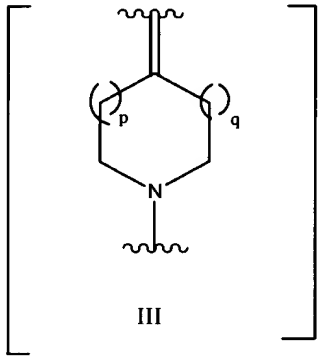


Formula I

M is a moiety having a general structure shown in Formula II[or III]:



II



III

where k = 0 or 1, n = 0-5, and p = q = 0, 1 or 2 [with the proviso that when M is Formula III, R^3 is absent];

V is a moiety selected from the group consisting of C_1-C_8 alkyl; $-(CH_2)_x-A-(CH_2)_y-$; and $-(CH_2)_c-A-(CH_2)_m-C(O)-N(R^7)-(CH_2)_d-$, where A is -O-, $-S(O)_r-$, and $-NR^7-$;

m = 0, 1, 2 or 3; x is a whole number in the range 2-8; y is a whole number in the range 1-5; c is a whole number in the range 2-4; and r= 0, 1 or 2; d is a number in the range 0-5;

X and Y are independently selected from the group consisting of N, and CH [, and N(O)];

Z and Z^1 can be the same or different, each being independently [is] selected from the group consisting of N, CH and N(O);

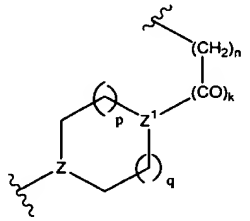
R^1 and R^2 may each number 1-4 and are independently selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, halogen, polyhalolower alkyl, polyhalolower alkoxy, -OH, CN, NO_2, or $COOR^8$;

R^3 is selected from hydrogen, lower alkyl, lower alkoxy, hydroxyl, with the proviso that when n and k are both 0, then R^3 is not -OH or alkoxy;

R^4 is selected from the group consisting of hydrogen, lower alkyl, polyhalolower alkyl or -OH; and

R^7 and R^8 are independently selected from hydrogen, lower alkyl, substituted or unsubstituted phenyl; and substituted or unsubstituted benzyl, wherein said term "substituted" means optional substitution from one or more moieties selected from the group consisting of alkyl, alkoxy, $-CF_3$, halogen or aryl.

Claim 4 (amended): The compound of claim 1, wherein [M is:



and] p and q are independently 0 or 1.

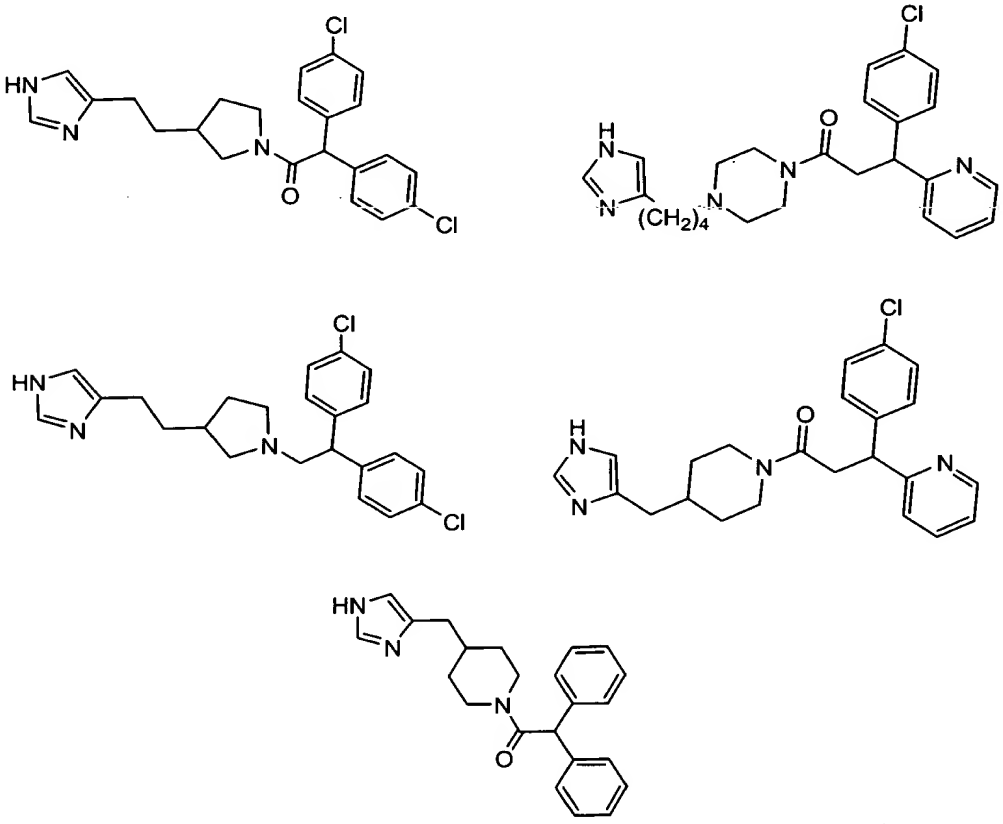
Claim 10 (amended): A pharmaceutical composition comprising as an active ingredient a compound of claim 1 and a pharmaceutically acceptable carrier.

Please cancel Claim 11 without prejudice.

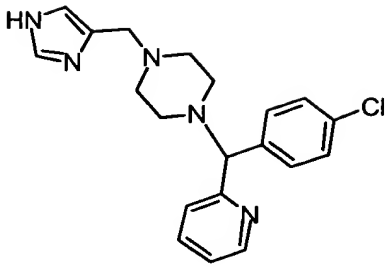
Please cancel Claim 12 without prejudice.

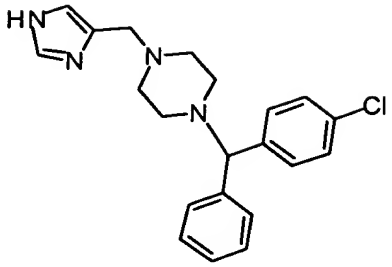
Please cancel Claim 14 without prejudice.

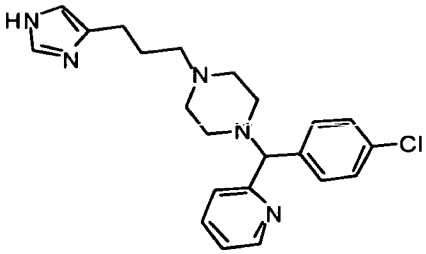
Claim 16 (amended): A compound exhibiting H_3 antagonist activity, or [including] enantiomers, stereoisomers and tautomers of said compound, or pharmaceutically acceptable salts or solvates of said compound, said compound being selected from the compounds with structures listed below:

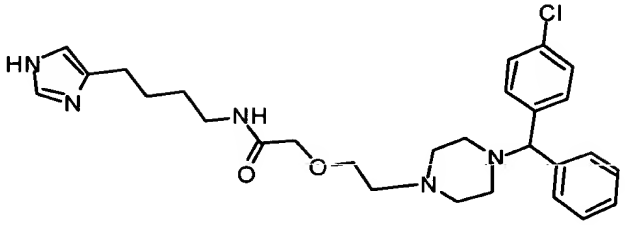


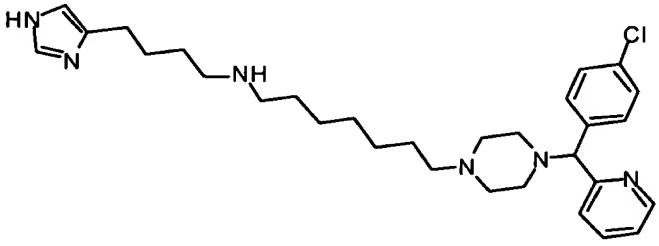
Claim 17 (amended): A compound exhibiting both H_1 and H_3 antagonist activity, or [including] enantiomers, stereoisomers and tautomers of said compound, or pharmaceutically acceptable salts or solvates of said compound, said compound being selected from the compounds with structures listed below:

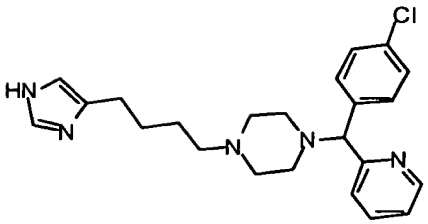


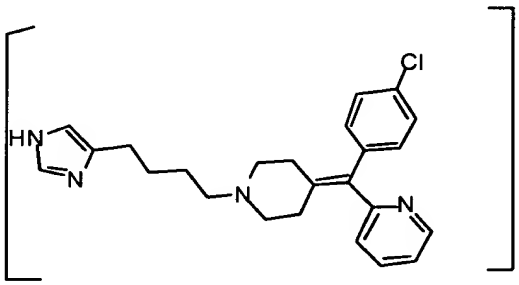


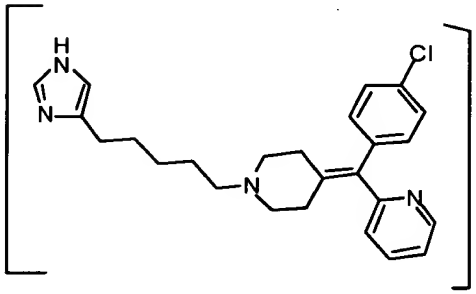


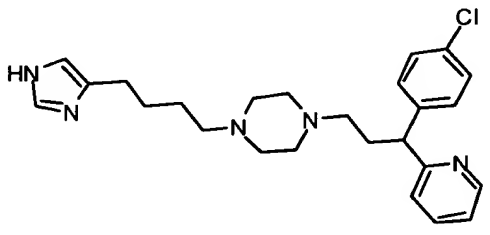


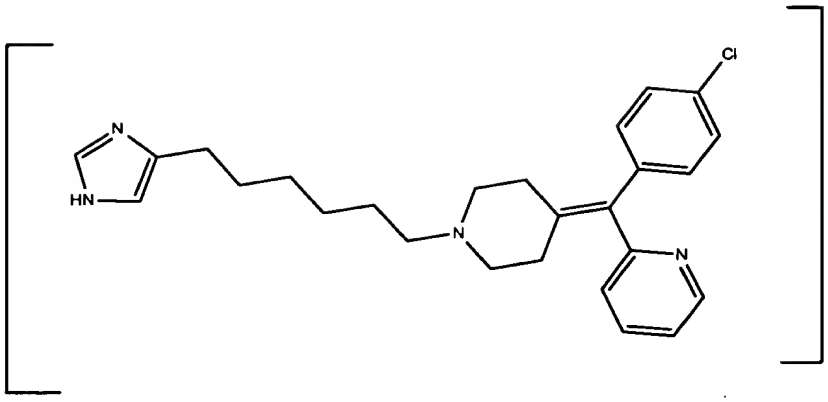


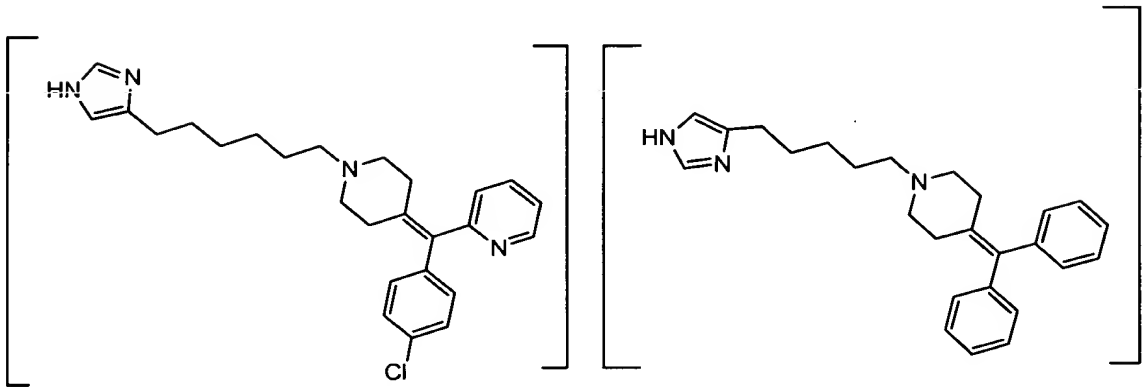


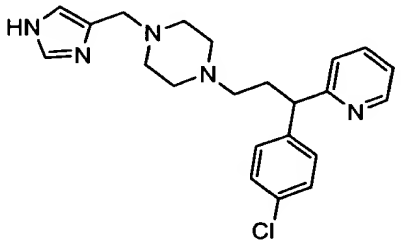


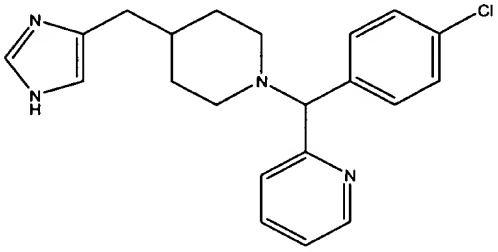












Claim 19 (New Claim): The compound of claim 4, wherein Z is N, Z^1 is CH, and p = q = 1.

Claim 20 (New Claim): The compound of claim 4, wherein Z^1 is N, Z is CH, and p = q = 1.

Claim 21 (New Claim): The compound of claim 20, wherein p = 1 and q = 0.

ORIGINAL ARTICLES

Institut für Pharmazie I[1], Freie Universität Berlin, Germany, Laboratoire Bioprojet[2], and Unité de Neurobiologie et Pharmacologie[3], Centre Paul Broca de l'INSERM, Paris, France

Search for novel leads for histamine H_3-receptor antagonists: oxygen-containing derivatives

H. Stark[1], A. Hüls[1], X. Ligneau[2], J.-M. Arrang[3], J.-C. Schwartz[3] and W. Schunack[3]

This study was performed in order to develop new leads for antagonists of the histamine H_3-receptor subtype. ω-(1 *H*-Imidazol-4-yl)alkyl derivatives with ester, ketone or alcohol functionality in the side chain were synthesized and tested concerning their H_3-receptor antagonist activity on synaptosomes of rat cerebral cortex. The novel compounds, which possess no nitrogen-containing polar group in the side chain of the imidazole moiety, presented moderate to high antagonist potency *in vitro*. In this series 3-(1 *H*-imidazol-4-yl)propyl-3-cyclopentylpropanoate (**4**) was the most potent compound *in vitro* with $-\log K_i = 8.5$. Unfortunately, no central antagonist H_3-receptor activity was detectable for ester derivatives in the *in vitro* H_3-receptor assay based upon measurement of brain N^{τ}-methylhistamine levels after p.o. administration to mice. Some of these novel antagonists are useful tools for investigations on ligand-receptor interaction because of their distinct receptor activities. On the other hand, the ketone derivative 1-(1 *H*-imidazol-4-yl)-7-phenyl-4-heptanone (**9**) *in vitro* presented an ED_{50}-value of 3.5 ± 1.5 mg/kg p.o. thus proving to be a new lead for further drug investigations. The most potent compounds *in vitro* and *in vivo* also showed high H_3-receptor selectivity when tested at other histamine receptor subtypes.

Suche nach neuen Leitstrukturen für Histamin-H_3-Rezeptorantagonisten: Sauerstoffhaltige Verbindungen

Diese Studie wurde mit dem Ziel durchgeführt, neue Antagonisten des Histamin-H_3-Rezeptorsubtyps zu entwicklen. ω-(1 *H*-Imidazol-4-yl)alkylderivate mit Ester-, Keton- oder Alkoholfunktion in der Seitenkette wurden synthetisiert und auf ihre antagonistische Aktivität an Histamin-H_3-Rezeptoren von Synaptosomen des Zerebralkortex der Ratte untersucht. Die neuen Verbindungen, die keine stickstoffhaltigen polaren Gruppen in der Seitenkette des Imidazolringes besitzen, zeigten moderate bis hohe Aktivität *in vitro*. 3-(1 *H*-Imidazol-4-yl)propyl-3-cyclopentylpropanoat (**4**) war mit $-\log K_i = 8{,}5$ *in vitro* die potenteste Verbindung dieser Serie. Unglücklicherweise waren die Esterderivate in einem *in vivo* H_3-Rezeptorassay basierend auf der Messung von N^{τ}-Methylhistaminspiegeln im Gehirn nach p.o. Verabreichung an Mäuse ohne zentrale antagonistische H_3-Rezeptoraktivität. Einige dieser neuen Antagonisten sind aufgrund ihrer hohen H_3-Rezeptoraktivität nützliche Verbindungen für weitere Untersuchungen von Ligand-Rezeptor-Wechselwirkungen. Demgegenüber zeigte das Ketonderivat 1-(1 *H*-Imidazol-4-yl)-7-phenyl-4-heptanon (**9**) einen *in vivo* ED_{50}-Wert von $3{,}5 \pm 1{,}5$ mg/kg p.o. Es stellt somit eine neue Leitstruktur für die weitere Arzneistoffentwicklung dar. Die potentesten Verbindungen *in vitro* und *in vivo* wiesen ebenfalls hohe H_3-Rezeptorselektivität auf, was durch Untersuchungen an anderen Histaminrezeptor-Subtypen belegt werden konnte.

1. Introduction

The discovery of the third histamine receptor subtype, the so-called histamine H_3-receptor, in 1983 by Arrang et al. [1] brought fresh impetus into an old field of research, and an intensive research programme was started to develop potent and selective ligands. These ligands should be useful tools to investigate the physiological and pharmacological functions of the histamine H_3-receptor. This receptor was first identified as an autoreceptor controllings its own release and synthesis *via* a negative feedback mechanism [1, 2]. In the meantime H_3-receptors were also characterized as heteroreceptors with modulating effects for a number of different neurotransmitters [2–7]. At present histamine H_3-receptor antagonists have not yet been submitted to clinical trials. Several therapeutic indications for H_3-receptor antagonists have been proposed for various diseases or conditions of the CNS [8, 9], e.g., memory and learning deficits [10, 11], cognitive and sleep disorders [12], and epilepsy [13].

During the last decade a large number of compounds containing different functionalities have been designed in different laboratories [14–24]. In a previous paper we newly described urea derivatives of ω-(1 *H*-imidazol-4-yl)-alkanamines as potent and selective H_3-receptor antagonists [25].

A general construction pattern of histamine H_3-receptor antagonists obtained by abstraction of all ligands [2, 8] is characterized by a nitrogen-containing heterocycle connected by an alkyl spacer to a polar moiety which is connected to a lipophilic residue directly or by another spacer. Since most of the histamine H_3-receptor antagonists possess nitrogen-containing polar groups the aim of this study was to develop new compounds structurally different from the known antagonists without nitrogen atoms in the polar group. The novel compounds possess ester, inverse ester, ketone and alcohol groups as polar functionalities in the alkyl side chain of the mono-4-substituted imidazole ring connected with different spacers and various lipophilic residues fitting to the general construction as shown in Fig. 1. Furthermore, the aim of this study was the finding, but not the optimization, of novel leads. The pharmacological evaluation concerning histamine H_3-receptor *in vitro* and *in vivo* activities and the determination of the selectivity of selected compounds were performed in this study.

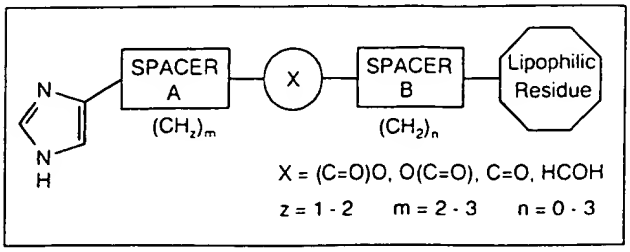


Fig. 1: General construction pattern for compounds 1–10

2. Investigations, results and discussion

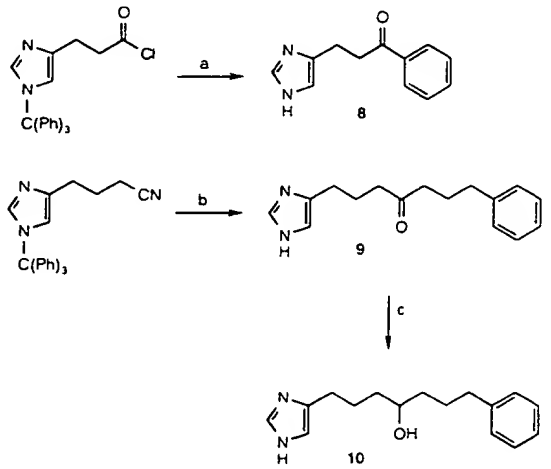
2.1. Chemistry

The novel compounds can be classified into four groups: esters (**1–5**), inverse esters (**6**, **7**), ketones (**8**, **9**), and alcohol (**10**). The esters **1–5** were prepared by Einhorn reaction [26] of carboxylic halids with 3-(1 *H*-imidazol-4-yl)propanol [20] in pyridine with addition of 4-(dimethylamino)-pyridine (DMAP) as a hyper nucleophilic acylating catalyst (Scheme 1). The diacylated by-product could be mono-hydrolyzed at the imidazole nitrogen by addition of water. The esters were separated and purified by chromatography. The inverse esters **6** and **7** were prepared by esterification of carboxylic compounds or reesterification with the corresponding alcohols under acid catalysis (Scheme 1). The ketone derivative **8** was prepared by Friedel-Crafts acylation of benzene with *N*-tritylated 3-(1 *H*-imidazol-4-yl)propanoyl chloride and $AlCl_3$ as Lewis acid (Scheme 2). Cleavage of the protecting group could be performed in almost quantitative yields by acidic hydrolysis. The ketone derivative **9** was synthesized by Grignard reaction of the corresponding nitrile with 3-phenylpropyl magnesium bromide (Scheme 2). The nitrile derivative was obtained from urocanic acid in a five step synthesis according to Sellier et al. [27]. Reduction of the ketone **9** by complex hydrides led to the corresponding racemic alcohol derivative **10** (Scheme 2).

2.2. Pharmacology

Histamine H_3-receptor antagonist activity of the compounds was determined by means of an *in vitro* test system on the basis of K^+-evoked depolarization induced release of [^{3}H]histamine from rat cerebral cortex synaptosomes according to Garbarg et al. [28] (Table 1). The *in vivo* testing was performed after peroral administration to Swiss mice on the brain histamine turnover being measured by determining the level of the main metabolite of histamine, N^{τ}-methylhistamine [28]. The affinity for other histamine receptors was tested for H_2-receptors at the isolated spontaneously beating guinea pig right atrium and for H_1-receptors at the isolated guinea pig ileum [29].

Scheme 2



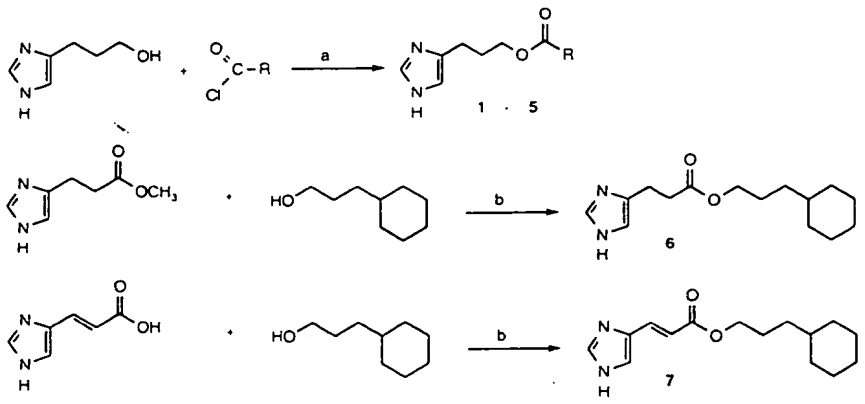
(a) i: benzene, $AlCl_3$; ii: 2 N HCl; iii: Na_2CO_3; iv: maleic acid; (b) i: 3-phenylpropyl magnesium bromide, Et_2O/THF; ii: NH_4Cl solution; iii: 2 N HCl; iv: NH_3; v: maleic acid; (c) i: $LiAlH_4$, Et_2O/dioxane; ii: NaOH/CH_2Cl_2; iii: maleic acid

2.2.1. In vitro results and discussion

All compounds belong to different alkyl substituted 4-imidazolyl derivatives. They all possess moderate to high histamine H_3-receptor antagonist activity except for the alkenyl substituted derivative **7**.

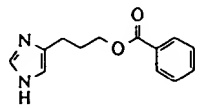
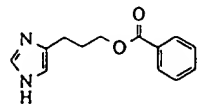
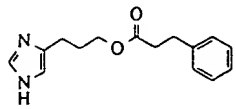
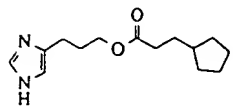
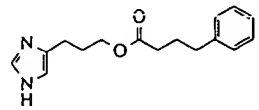
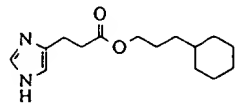
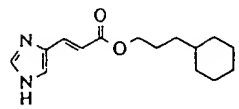
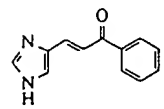
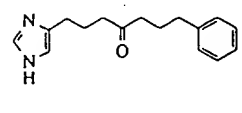
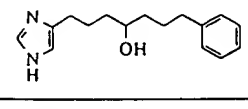
Ester derivatives of 3-(1 *H*-imidazol-4-yl)propanol showed high antagonist potency. In a related amide series [19] and also in an ester series prepared in search of [^{125}I]iodinated radioligands [20] three methylene groups for spacer A were found to be the optimum. Therefore, for this study sapcer A was modified only to a small extent. The structure of the esters could be optimized by variation of the spacer between ester and lipophilic moiety (**1–3**, **5**). Spacer B with two methylene groups was the optimum in this series of antagonists (**3**, **4**). Similar structure-activity relationships were established for a related amide series [19]. Therefore, the 3-cyclopentylpropanoic acid derivative **4** was prepared, this moiety being one of the best for re-

Scheme 1



(a) i: DMAP, pyridine; ii: H_2O, 2 N NaOH; iii: maleic acid; (b) i: gaseous HCl; ii: K_2CO_3; iii: maleic acid

Table 1: Structures and histamine H_3-receptor antagonist activity *in vitro* and *in vivo*

Compd.	Structure	K_i ($\bar{x} \pm s_{n-1}$) (nM)	$-\log K_i$	ED_{50} ($\bar{x} \pm s_{n-1}$) (mg/kg)
1		74 ± 22	7.1	> 10
2		43 ± 6	7.4	> 10
3		15 ± 3[a]	7.8	> 10
4		2.9 ± 0.3[b]	8.5	> 10
5		33 ± 12	7.5	> 10
6		140 ± 30	6.9	> 10
7		> 530	< 6.3	> 10
8		390 ± 117	6.4	> 10
9		23 ± 5	7.6	3.5 ± 1.5
10		111 ± 70	7.0	≈ 10

[a] ref. [23] (pK_i = 8.1 binding experiments, rat brain cortex membranes; pA_2 = 7.8 functional experiments, mice brain cortex slices); [b] ref. [21, 35] (pK_i = 8.5 binding experiments, rat brain cortex membranes; pA_2 = 8.3 functional experiments, mice brain cortex slices)

lated amides [19]. The structure-activity relationships could be confirmed by the test results of **4**. Compound **4** is about two times more potent than the reference antagonist thioperamide (K_i = 4.3 nM [15]) and about 5 times more than the related phenyl derivative **3**. With an inverse ester structure the related compound **6** also showed moderate histamine H_3-receptor antagonist potency, but its activity is about 48 times lower than that of compound **4**. The related alkenyl derivative **7** does not show detectable affinity for histamine H_3-receptors. Due to the low affinity of the derivative of urocanic acid (**7**), this compound in association with the other ester derivatives is a useful tool for computational modeling investigation on ligand-receptor interactions.

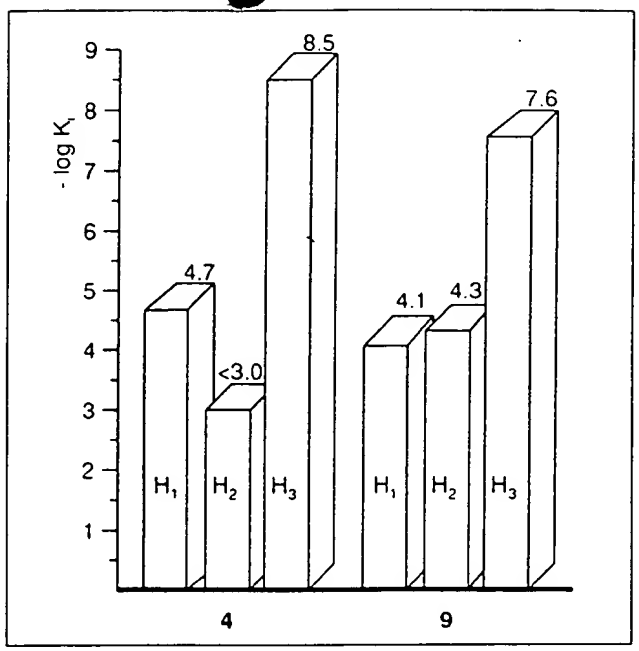


Fig. 2: Antagonist activity of compounds **4** and **9** at histamine receptor subtypes

The ester moiety is not the only functionality without a nitrogen atom that can be introduced by maintaining histamine H_3-receptor antagonist activity. Ketone derivative **8** showed moderate affinity which could be improved by introduction of a spacer B leading to compound **9** being as active as the comparable ester **3**. The carbonly moiety is not an essential element for histamine H_3-receptor antagonists as shown with the alcohol derivative **10**. Although **10** is five times less active than the corresponding carbonyl derivative **9** it showed remarkable H_3-receptor activity. With the ester, ketone and alcohol series three novel classes of histamine H_3-receptor antagonists could be established.

2.2.2. *In vivo results and discussion*

Whereas almost all compounds showed histamine H_3-receptor antagonist activity under *in vitro* conditions, only ketone derivative **9** and the related alcohol **10** showed detectable central histamine H_3-receptor activity after p.o. administration to mice under *in vivo* conditions. All other compounds like esters **1–5**, inverse ester **6** and **7**, as well as ketone **8** showed no measurable *in vivo* activity under these screening conditions. The reason for this loss in activity might be pharmacokinetic. In the case of compounds **1–5** ester hydrolysis by unspecific esterases perhaps leads to the inactive 3-(1*H*-imidazol-4-yl)propanol (ED_{50} > 10 mg/kg [20]), or in the case of **6** and **7** to 3-cycloalkylpropanol. This hypothesis is strengthened by the observation that ketone **9** and the alcohol derivative **10** without functionalities for esterases hydrolysis showed detectable activity *in vivo*. Although its *in vivo* activity is lower than that of the reference antagonist thioperamide (ED_{50} = 1 ± 0.5 mg/kg [28]) compound **9** presents a promising lead for the further developments without sulfur moieties which are claimed to be responsible for thioperamide's (hepato)toxicity. Isothiourea derivatives like clobenpropit showed comparable difference to esters for *in vitro* and *in vivo* activity. Clobenpropit is also highly active *in vitro* [17], but had low *in vivo* activity after peroral application (ED_{50} = 26 ± 7 mg/kg [20]).

Table 2: Analytical data of compounds 1–10

Compd. (m.p. °C)	Formula (molecular weight)	MS m/e (rel. int. %)	^{1}H NMR (δ in ppm)
1 (102)	$C_{13}H_{14}N_2O_2 \cdot C_4H_4O_4 \cdot 0.5\,H_2O$ (355.3)	230 (8[M]$^{+\cdot}$), 95(100)	8.89 s, 1 H, Im–2-H; 7.96–7.46 m, 5 H, Phe; 7.46 s, 1 H, Im–5-H; 6.05 s, 2 H, Mal; 4.31 t, J = 6.0 Hz, 2 H, O–CH_2; 2.81 t, J = 7.4 Hz, 2 H, Im–CH_2; 2.08 m, 2 H, Im–CH_2–CH_2
2 (116)	$C_{14}H_{16}N_2O_2 \cdot C_4H_4O_4$ (360.4)	244 (18 [M]$^{+\cdot}$), 95(100)	8.89 s, 1 H, Im–2-H; 7.38 s, 1 H, H–C-5 Im; 7.35–7.25 m, 5 H, Phe; 6.05 s, 2 H, Mal; 4.06 t, J = 6.4 Hz, 2 H, O–CH_2; 3.66 s, 2 H, CH_2–Phe; 2.67 t, J = 7.0 Hz, 2 H, Im–CH_2; 1.92 m, 2 H, Im–CH_2–CH_2
3 (112)	$C_{15}H_{18}N_2O_2 \cdot C_4H_4O_4$ (374.4)	258 (11 [M]$^{+\cdot}$), 95(100)	8.88 s, 1 H, Im–2-H; 7.38 s, 1 H, Im–5-H; 7.30–7.18 m, 5 H, Phe; 6.04 s, 2 H, Mal; 4.02 t, J = 6.3 Hz, 2 H, O–CH_2; 2.84 t, J = 7.6 Hz, 2 H, Im–CH_2; 1.89 m, 2 H, Im–CH_2–CH_2
4 (88)	$C_{14}H_{22}N_2O_2 \cdot C_4H_4O_4$ (366.4)	250 (8 [M]$^{+\cdot}$), 95(100)	8.88 s, 1 H, Im–2-H; 7.41 s, 1 H, Im–5-H; 6.05 s, 2 H, Mal, 4.04 t, J = 6.4 Hz, 2 H, O–CH_2; 2.69 t, J = 7.5 Hz, 2 H, Im–CH_2; 2.28 t, J = 7.6 Hz, CH_2–C=O; 1.92 m, 2 H, Im–CH_2–CH_2; 1.71–1.06 m, 11 H, 5 CH_2 1 CH
5 (88)	$C_{16}H_{20}N_2O_2 \cdot C_4N_4O_4 \cdot 0.5\,H_2O$ (397.4)	272 (31 [M]$^{+\cdot}$), 95(100)	8.87 s, 1 H, Im–2-H; 7.41 s, 1 H, Im–5-H; 7.31–7.17 m, 5 H, Phe; 6.05 s, 2 H, Mal; 4.04 t, J = 6.3 Hz, 2 H, O–CH_2; 2.68 t, J = 7.6 Hz, 2 H, Im–CH_2; 2.58 t, J = 7.6 Hz, 2 H, C=O–CH_2; 2.29 t, J = 7.3 Hz, 2 H, CH_2–Phe; 1.92 m, 2 H, CH_2–CH_2–Phe; 1.81 m, 2 H, Im–CH_2–CH_2
6 (126)	$C_{15}H_{24}N_2O_2 \cdot C_4H_4O_4 \cdot 0.5\,H_2O$ (389.5)	264 (6 [M]$^{+\cdot}$) 95(100)	8.85 s, 1 H, Im –2-H; 7.38 s, 1 H, Im–5-H; 6.05 s, 2 H, Mal, 3.97 m, 2 H, O–CH_2; 2.88 t, J = 7.3 Hz, C=O–CH_2; 2.71 t, J = 7.2 Hz, 2 H, Im–CH_2; 1.67–0.81 m, 15 H, 7 CH_2, CH
7 (149)	$C_{15}H_{22}N_2O_2 \cdot C_4H_4O_4$ (378.4)	262 (22 [M]$^{+\cdot}$), 121(100)	8.29 s, 1 H, Im–2-H; 7.74 s, 1 H, Im–5-H; 7.54 d, J = 15.9 Hz, 1 H, CH–CO; 6.46, d, J = 15.9 Hz, 1 H, Im–CH; 6.19 s, 2 H, Mal; 4.10 t, J = 6.6 Hz, 2 H, O–CH_2; 1.71–0.84 m, 15 H, 7 CH_2, CH
8 (152)	$C_{12}H_{12}N_2O \cdot C_4H_4O_4$ (316.3)	200 (4 [M]$^{+\cdot}$), 95 (100)	8.86 s, 1 H, Im–2-H; 8.01–7.53 m, 5 H, Phe; 7.40 s, 1 H, Im–5-H; 6.04 s, 2 H, Mal; 3.49 tr, J = 7 Hz, 2 H, C=O–CH_2; 2.98 tr, J = 7 Hz, 2 H, Im–CH_2
9 (129)	$C_{16}H_{20}N_2O \cdot C_4H_4O_4 \cdot 0.5\,H_2O$[a] (381.4)	256 (2 [M]$^{+\cdot}$), 95(100)	8.89 s, 1 H, Im–2-H; 7.40 s, 1 H, Im–5-H; 7.35 d, J = 7.8 Hz, 2 H, H–C-2 Phe, H–C-6 Phe; 7.19 m, 3 H, H–C-3 Phe, H–C-4 Phe, H–C-5 Phe; 6.05 s, 2 H, Mal; 2.73–2.39 m, 8 H, Im–CH_2, CH_2–Phe, CH_2–O–CH_2; 1.75 m, 4 H, 2 CH_2
10 (87)	$C_{16}H_{22}N_2O \cdot C_4H_4O_4\,H_2O$ (392.5)	259 (100 [M]$^{+\cdot}$)	8.85 s, 1 H, Im–2-H; 7.36 s, 1 H, Im–5-H; 7.26 d, J = 6.8 Hz, 2 H, H–C-2 Phe, H–C-6 Phe; 7.19 m, 3 H, H–C-3 Phe, H–C-4 Phe, H–C-5 Phe; 6.03 s, 2 H, Mal; 3.46 m, 1 H, CH–O; 2.54 m, 4 H, Im–CH_2, CH_2–Phe; 1.67–1.24 m, 8 H, 4 CH_2

[a] N calc. 7.34, found 7.86

2.2.3. *Histamine receptor subtype selectivity*

Compounds **4** and **9**, most potent *in vitro* and *in vivo*, were tested on isolated organs of guinea pig for their affinity to other histamine receptors (Fig. 2). The result thereof was an affinity at least 2000 times less than at H_3-receptors demonstrating the high selectivity of these compounds.

It can be concluded that the novel compounds without a nitrogen atom in the side chain of the mono-4-substituted imidazole moiety, except for the inverse alkenyl ester **7**, i.e., esters **1–5**, inverse ester **6**, ketones **8** and **9**, as well as the alcohol **10** represent histamine H_3-receptor ligands with moderate to high antagonist potency. Different classes of new antagonists could be established. The importance of chain B of the general construction pattern could be shown for esters' *in vitro* and ketones' *in vivo* activity. The highest potencies *in vitro* were observed for ester derivative **4** and *in vivo* for ketone derivative **9**. Compounds **4** and **9** showed only low affinity to H_1- and H_2-receptors proving their selectivity. Therefore, ketone derivative **9** proved to be a novel lead for potential drugs possessing *in vitro* as well as *in vivo* H_3-receptor antagonist actitivity and high selectivity.

3. Experimental

3.1. *Chemistry*

M.p.'s were determined on an Electrothermal IA 9000 digital apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 300 (300 MHz) spectrometer using D_6-DMSO as solvent. Chemical shifts (δ) are expressed in ppm downfield from internal TMS as reference (Im: imidazolyl, Mal: maleic acid, d: doublet, s: singlet, tr: triplet). MS were obtained on an EI-MS Finnigan MAT CH7A and a Finnigan MAT 711 (for all data see Table 2). Elemental analyses (C, H, N) were measured on Perkin-Elmer 240 B or Perkin-Elmer 240 C instruments, and were within ±0.4% of the theoretical values unless otherwise mentioned. TLC was performed on silica gel PF_{254} plates (Merck). Preparative TLC was performed with a Chromatotron 7924T (Harrison Research) with glass rotors with 4 mm layers of silica gel 60 PF_{254} containing gypsum (Merck), or by CC using silica gel 62–200 µm (Macherey, Nagel & Co.).

3.1.1. *General procedure for the synthesis of esters 1–5*

The corresponding carboxylic acid (5 mmol) was stirred with 30 ml thionyl chloride for 12 h at ambient temperature. The solvent was evaporated, and the acyl chloride was dissolved in dry pyridine. To this solution 3-(1*H*-Imidazol-4-yl)propanol hydrochloride (0.8 g, 5 mmol [30]) and a catalytic amount of DMPA were added, and the reaction mixture was refluxed for 4–8 h. The solvent was evaporated under reduced pressure, and the oily residue was dissolved in 10 ml of H_2O. The aqueous layer was alkalized with 2 N NaOH and extracted with ethyl acetate. The organic extract was washed with H_2O and dried over Na_2SO. After removal of the solvent

under reduced pressure the residue was purified by silica gel CC using CH_2Cl_2/MeOH/NH_3 (25%) (80:19:1). This yielded a colourless oil which was crystallized as hydrogen maleate from CH_3CN/diethyl ether.

3.1.2. Synthesis of ester 6

Methyl 3-(1 *H*-imidazol-4-yl)propanoate hydrochloride (0.9 g, 5 mmol [27]) and 20 ml 3-cyclohexylpropanol were refluxed. Dry HCl was introduced for 1 h while reflux was continued. The mixture was added to 100 ml of ethyl acetate and washed with K_2CO_3 solution and H_2O. The organic layer was evaporate under reduced pressure, and the resulting oil was crystallized as hydrogen maleate and recrystallized with CH_3CN/diethyl ether.

3.1.3. Synthesis of ester 7

3-(1 *H*-Imidazol-4-yl)propenic acid (urocanic acid, 1.4 g, 10 mmol) was treated with 3-cyclohexylpropanol as described above for **6**.

3.1.4. Synthesis of ketone 8

3-((1-Triphenylmethyl)-1 *H*-imidazol-4-yl)propanoyl chloride hydrochloride (freshly prepared from 5 mmol 3-((1-triphenylmethyl)-1 *H*-imidazol-4-yl)propanoic acid [27] with equimolar amounts of $SOCl_2$ in tetrahydrofurane) was suspended in 150 ml of benzene with Argon protection against moisture. With ice cooling $AlCl_3$ (20 mmol) was slowly added and stirred for 2 h. After keeping the solution 3 h under reflux it was stirred overnight. The solution was evaporated to dryness, dissolved in 2 N HCl and ethanol and kept under reflux for 3 h. Ethanol was evaporated, and triphenylmethanol was filtered off. Traces of impurities were extracted with diethyl ether. The solution was alkalized with Na_2CO_3 and evaporated to total dryness. The residue was extracted several times with hot ethyl acetate. The combined organic layers were evaporated to yield a yellow oil which was crystallized as hydrogen maleate and recrystallized with C_2H_5OH/diethyl ether.

3.1.5. Synthesis of ketone 9

4-(1-Triphenylmethyl-1 *H*-imidazol-4-yl)butylnitrile (1.9 g, 2 mmol [27]) was given into a mixture of 3-phenylpropan magnesium bromide (2 mmol, freshly prepared) in 300 ml diethyl ether and 100 ml dry tetrahydrofurane. The solution was refluxed for 5 h. The mixture was cooled and hydrolyzed with a solution of NH_4Cl. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. Thereafter the combined organic layers were heated to reflux for 2 h in a solution of 50 ml of C_2H_5 OH and 50 ml 2 N HCl. The solvent was evaporated under reduced pressure, the cooled solution was filtered and washed with diethyl ether. The aqueous layer was alkalized with NH_3 and extracted with CH_2Cl_2. The combined organic solutions were dried, and the solvent was evaporated. The residue was crystallized as hydrogenmaleate and recrystallized with C_2H_5OH/diethyl ether.

3.1.6. Synthesis of alcohol 10

Ketone **9** (0.5 g, 2 mmol) was stirred in a mixture of $LiAlH_4$ (0.3 g, 0.8 mmol), 30 ml diethyl ether, and 10 ml dioxane at ambient temperature for 12 h. Thereafter the mixture was hydrolized with 1 N NaOH. The residue was extracted with CH_2Cl_2. The organic layer was evaporated, the residue was dissolved in dry C_2H_5OH and evaporated to dryness. The resulting oil was crystallized as hydrogen maleate and recrystallized with C_2H_5OH/diethyl ether.

3.2. Pharmacology

3.2.1. Histamine H_3-receptor in vitro assay on synaptosomes of rat cerebral cortex

The presented compounds were tested for their H_3-receptor antagonist activity in an assay with K^+-evoked depolarization-induced release of [^{3}H]histamine from synaptosomes of rat cerebral cortex according to Garbarg et al. [28]. A synaptosomal fraction from rat cerebral cortex prepared according to the method of Whittaker [31] was preincubated for 30 min with L-[^{3}H]histidine (0.4 µM) at 37 °C in a modified Krebs-Ringer solution. Then the synaptosomes were washed extensively, resuspended in fresh 2 mM of K^+ Krebs-Ringer's medium, and incubated for 2 min with 2 or 30 mM K^+ (final concentration). Drugs and 1 µM of histamine were added 5 min before the depolarization stimulus. Incubations were stopped by rapid centrifugation, and [^{3}H]histamine levels were determined after purification by liquid scintillation spectrometry [28]. K_i values were determined according to Cheng-Prusoff equation [32]. The data presented are given as mean values with standard error of the mean for a minimum of three different determinations each.

3.2.2. Histamine H_3-receptor antagonist in vivo activity in mice

Increase in level of the main metabolite of histamine, N^π-methylhistamine, in Swiss mice brain after p.o. application of compounds **1**–**10** was selected to determine histamine H_3-receptor antagonist *in vivo* activity. Mice were fasted for 24 h before p.o. treatment. Animals were decapitated 90 min after treatment, and the brain was dissected out and homogenized in 10 vol of icecold $HClO_4$ (0.4 M). The N^π-methylhistamine level was measured by a radioimmunoassay described by Garberg et al. [33]. Through treatment with 10 mg/kg of thioperamide the maximal N^π-methylhistamine level was obtained and related to the level reached with the administered drug. ED_{50} values were calculated based on the weight of the free base [34].

3.2.3. In vitro screening at other histamine receptor subtypes

Compounds **4** and **9** were tested for their histamine H_2-receptor activity at isolated spontaneously beating guinea pig right atrium as well as for histamine H_1-receptor activity at isolated guinea pig ileum by standard methods according to Hirschfeld et al. [29]. Each pharmacological test was performed in triplicate, but the exact type of receptor interaction was not determined in each case. The given values represent the mean.

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Synthesis of novel ring systems: Synthesis of imidazo[1,2-*c*]-1,2,4-triazolo[4,3-*a*]pyrimido[5′,4′:4,5]thieno[2,3-*e*]pyrimidine derivatives

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A series of partially hydrogenated imidazo[1,2-*c*]pyrimido[5′,4′:4,5]thieno[2,3-*e*]pyrimidines (**5–11**) have been synthesized from the *o*-aminonitrile **3** through the reaction with ethylene diamine and subsequent condensation of the resulting intermediate **4** with orthoester, carbon disulfide and aldehydes. The triazolo derivatives **13–16** were achieved through the reaction of the hydrazino compound **12** with reagents such as acetic anhydride, ethyl chloroformate and carbon disulfide.

Synthese neuer Ringsysteme: Synthese von Imidazo[1,2-*c*]-1,2,4-triazolo[4,3-*a*]pyrimido[5′,4′:4,5]thienol[2,3-*e*]pyrimidin-Derivaten

Eine Reihe partiell hydrierter Imidazo[1,2-*c*]pyrimido[5′,4′:4,5]thieno[2,3-*e*]pyrimidine (**5–11**) wurde ausgehend von dem Aminonitril **3** durch Reaktion mit Ethylendiamin und nachfolgende Kondensation des erhaltenen Zwischenproduktes **4** u. a. mit Orthoameisensauretriethylester, Schwefelkohlenstoff und Aldehyden dargestellt. Die Triazoloderivate **13–16** wurden durch Umsetzung der Hydrazinoverbindung **12** mit Reagentien wie Acetanhydrid, Chlorameisensäureethylester und Schwefelkohlenstoff erhalten.

1. Introduction

The synthesis of the imidazole ring via the reaction of *o*-aminonitrile with alkanediamines has been investigated by several groups [1–3]. Thus due to the high biological activity of imidazopyrimidines [4, 5], and thienopyrimidines [6, 7] and in continuation of our strategy in the use of nitriles for the synthesis of condensed heterocyclic systems [8, 9], it seemed desirable to apply this rection for the synthesis of heterocyclic compounds containing these ring systems and evaluate the compounds as bactericides and fungicides.

2. Investigations, results and discussion

2.1. Chemistry

The starting 5-cyano-2,6-diphenylpyrimidine-4(3*H*)thione (**1**) was readily obtained by a previously described procedure [10]. Compound **1** was reacted with chloroacetonitrile in the presence of anh. sodium acetate to give the cyanomethylthio derivative **2**, which in turn was cyclized into 3-amino-2-cyano-4,6-diphenylthieno[2,3-*d*]pyrimidine (**3**) by the action of ethanolic sodium ethoxide. The *o*-aminonitrile **3** was allowed to react with ethylene diamine in the presence of a catalytic ammount of carbon disulfide to afford the 2-imidazolyl derivative **4**. The IR spectrum of **4** revealed the disappearance of absorption at 2220 cm^{-1} (CN group).
Compound **4** was used as a key intermediate to synthesize tetracyclic derivatives containing the imidazopyrimidine moiety. Thus, the 2-imidazo derivative was condensed with triethyl orthoformate in the presence of a few drops of acetic acid to give the pyrimidine derivative **5**, while when refluxed in acetic anhydride, the 2-methyl derivative **6** was obtained.
Compound **4** was allowed to react with ethyl chloroformate and carbon disulfide to afford the pyrimidine-2-(1*H*)one **7** and the pyrimidine-2(1*H*)thione **8**, respectively. Compound **8** was easily S-alkylated with α-haloketones, α-haloesters, and α-halonitriles to give compounds **9a–f** (Scheme 1). Also, compound **4** was condensed with aromatic aldehydes in acetic acid to give 2-arylpyrimidine derivatives **10a, b**. Diazotization of **4** with sodium nitrite in acetic acid/HCl mixture gave the triazine derivative **11**.
On the other hand, the pyrimidine-2(1*H*)thione **8** was transformed into the corresponding 2-hydrazino derivative **12** through the reaction with hydrazine hydrate in pyridine. As a part of our study which has designed to investigate pentacyclic heterocycles containing the triazole moiety, it became important to use this hydrazino derivative to prepare a series of new triazolo derivatives.
The new ring system triazoloimidazopyrimidothienopyrimidine **13** was obtained through the condensation of **12** with triethyl orthoformate in presence of a few drops of acetic acid. The 2-methyl derivative **14** was achieved by refluxing **12** with acetic anhydride. Other new derivatives of triazolo compounds **15** and **16** were synthesized from the hydrazino compound via the reaction with ethyl chloroformate or carbon disulfide in pyridine, respectively.